

Transfer of Toxic Algal Substances in Marine Food Chains¹

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ABSTRACT: Alcoholic and ether extracts of obligate herbivores, omnivores, and detritus feeders common on *Caulerpa* or in its communities were found, via comparative, and sometimes quantitative, thin-layer chromatography, to contain varying amounts of caulerpicin, caulerpin, palmitic acid, and β -sitosterol or to lack them. *Cerithium* and soft corals, which may be either omnivores or carnivores, on occasion contain caulerpicin. The crustacean detritus feeders did not seem to preserve either caulerpicin or caulerpin. It seems well demonstrated that caulerpicin and caulerpin, which, as produced by *Caulerpa*, are physiologically active and toxic to rats and mice, respectively, are transferred along the food chains and concentrated in the process at least in some herbivores.

CAULERPICIN (Doty and Santos, 1966) and caulerpin (Santos and Doty, 1968) have been described from various species in the genus *Caulerpa*, a seaweed of the algal phylum, Chlorophyta. The same species also consistently contain palmitic acid and β -sitosterol which are not physiologically active, but on the other hand these two new compounds are toxic or otherwise physiologically active in mice and rats (unpublished results obtained in cooperation with Dr. George W. Read and Mr. Midori Kashiwagi, University of Hawaii Medical School). Other algae are suspected of producing toxins that become concentrated in fish (Banner et al., 1963; Helfrich, Piyakarnchana and Miles, 1968) and thus may cause the fish-poisoning syndrome ciguatera.

Oxynoe panamensis, a sacoglossid mollusk found feeding on *Caulerpa* near La Paz, Baja California, has been described (Lewin, 1970, accompanying paper) as producing an exudate toxic to fish. Elsewhere such herbivorous opisthobranchiate sacoglossan gastropods are regularly described (e.g., Macnae, 1954; Burn,

1960; Gonor, 1961; Keen and Smith, 1961; Kay, 1964; Burn, 1966) as being in a rather close or obligate food relationship with *Caulerpa*, and the authors cited variously illustrate and describe the peculiar feeding apparatus, as well as co-host distribution of these animals. Further in this vein, one chemist, while working with caulerpicin on the present project, became sensitized to substances in crabs and shrimp taken as food from the general area where *Caulerpa* occurs in abundance.

As general information, there is an abundance of detritus feeders such as crabs and shrimp, omnivores or carnivores such as soft corals, and mud-dwelling omnivorous gastropods of the genus *Cerithium* in the natural and cultivated *Caulerpa* communities in the Philippines. In this latter connection, during 1967 Mr. Simplicio Berame, the principal *Caulerpa* pond operator there, showed the senior author damage to the commercial *Caulerpa racemosa* var. *uvifera* crop which he attributed to *Cerithium*. This damage was evidenced by scars or calluses at the ends of unusually short branchlets, or ramulus stubs devoid of their normal spheroidal tips.

Thus, it was decided to test the hypothesis that the chemical products of *Caulerpa* are variously concentrated or used by the animals which obtain their sustenance in the *Caulerpa* community. This work was initiated in the field by collecting and preserving the above-mentioned kinds of living organisms by drying them or by dropping them into ethanol.

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METHODS AND MATERIALS

Collections of *Cerithium coralium* (Kiener) were made from ponds formed of mangrove mud for the purpose of raising *Caulerpa* on the island of Mactan in the central Philippines. The animal was found only in very small numbers on the alga during the daytime visits to the area. Thus, collections (20276,⁴ 20277, 20278) were made of this animal, respectively, from the mud beneath the fronds of *C. racemosa* var. *uvifera* (20272) and *C. lamourouxii* (25299) and from the bottom of a new pond that had as yet no *Caulerpa* in it. The gastropods were broken in the field and the flesh picked out and dried before being transported to the laboratory. In the laboratory the remaining shell material was removed before extraction to reduce contamination, for example, by blue-green algae that might be growing on the shell surfaces.

One of the major pests in the Philippine *Caulerpa* farms is a shrimp (20578), probably *Alpheus euprosyne*, of which about 20 specimens, placed alive in alcohol, filled a half-liter bottle. Similarly, collections (20579) of five species of crabs (*Thalamita crenata*, *T. spinimana*, *Epixanthus dentatus*, *Uca annulipes*?, and *U. dussumieri*) were made and preserved in alcohol. The crabs and shrimp were associated with *Caulerpa racemosa* var. *uvifera* (20272) and were not far from *C. lamourouxii* (25299) in the same ponds on the shore of Mactan Island used as a source of the above *Cerithium*.

Soft corals of three kinds (not as yet identified) were collected on Luzon Island in the Philippines and preserved in alcohol. One of these (20451) was on *Caulerpa lentillifera* (20450) obtained at Matabungkay and the other two (20576, 20577) from the same *Caulerpa* species (20490) at Bulusan.

Oxynoe panamensis (20541) was obtained from mats of unusually delicate *Caulerpa sertularioides* (20540) growing near La Paz, Baja California, and immediately preserved in 95 percent alcohol.

All the field collections of the different animals and dried collections of their host *Caulerpa*

species were transported to Honolulu. In Honolulu the wet-preserved animals were extracted further with fresh 95 percent alcohol. The pooled alcoholic extract was evaporated, and the residue was taken up with ether and concentrated for comparative chromatography along with similar concentrated ether extracts of the related dry *Caulerpa* collections.

The comparative chromatography was done on thin-layer (=TLC) plates of Camag silica gel G with the four pure substances caulerpicin, caulerpin, palmitic acid, and β -sitosterol. The mobile phase routinely used was an ether-hexane-acetic acid (50:50:1) mixture. The results were the same whether the dried residue from alcoholic extracts of wet alcoholic materials was taken up in ether and used, or whether the specimens were dried in the field and ether-extracted in Honolulu without the alcohol step. All TLC's were made several times and with slight variations to clarify the particular results or assure tenability of the interpretations being made.

In order to obtain estimates of the concentrations of the different *Caulerpa* compounds, the alcoholic extracts were adjusted to the same ratio of dry weight of organism to extract volume. Equivalent amounts of the extracts were then preparatively chromatogrammed on a uniform set of plates on one of which a comparative TLC was run (Fig. 1) to assure the identity of the streaks as revealed in iodine vapor. The TLC streaks were marked; the iodine was allowed to evaporate; the material in the marked areas was scraped off; the chemicals were taken up in ether; and, after evaporating to dryness, the residues were weighed. Thus, the amount of crude substance in terms of percent crude yield of dry weight of source organism could be calculated.

RESULTS AND DISCUSSION

The relative abundance of the chemical contents of the animals (Table 1) is to be considered in relationship to the relative abundance of the same chemicals in the species of *Caulerpa* from the same community. All the species of *Caulerpa* of concern here had the same relative amounts of the four substances except the *Caulerpa racemosa* variety which contained but

⁴ Such 5-place numbers identify the voucher specimens among the senior author's and the Smithsonian Institution's collections.

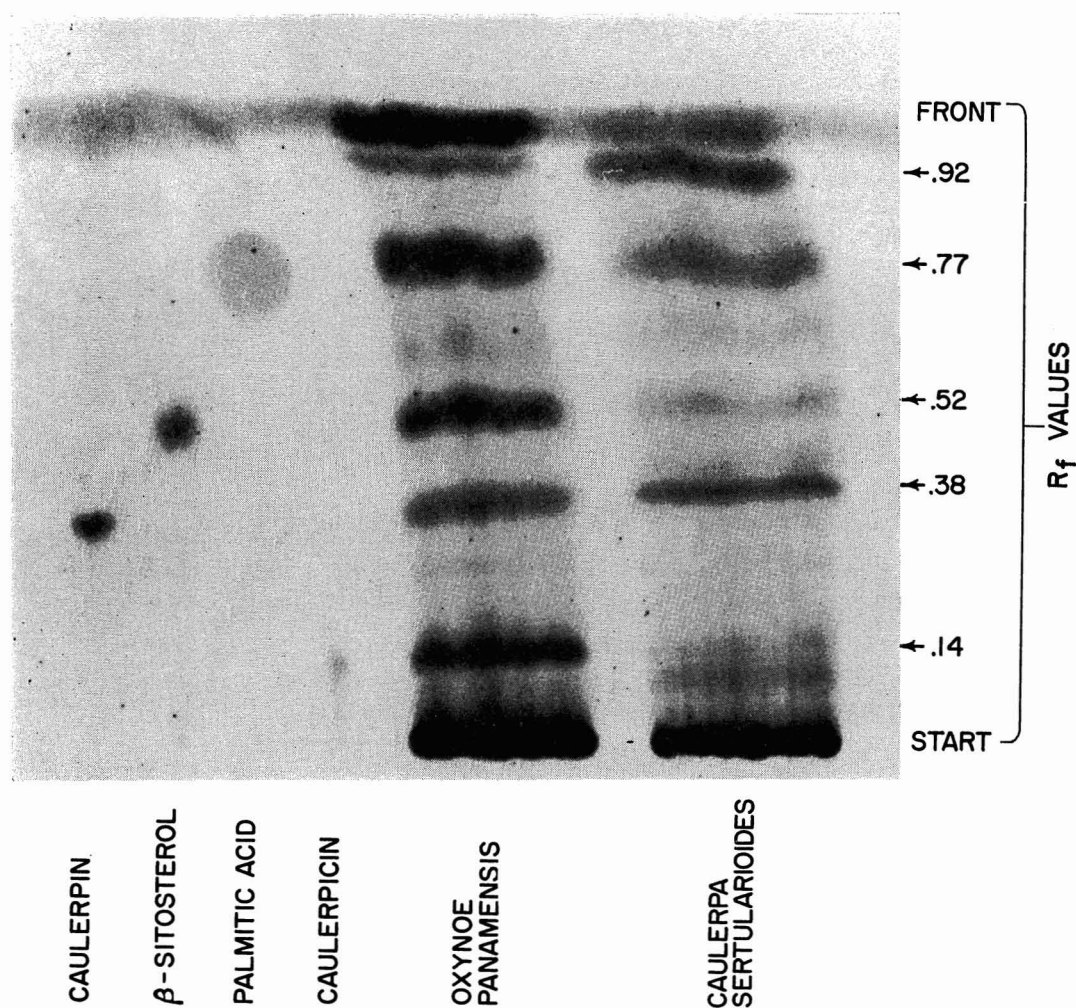


FIG. 1. Preparative thin-layer chromatogram of the ether extract from *Oxynoe panamensis* (20541) and *Caulerpa sertularioides* (20540), with a comparative chromatogram of the four pure or authentic substances on the left. The solvent used on this Camag silica gel G chromatogram was a 50:50:1 solution of ether, hexane, and acetic acid.

traces of caulerpin and no caulerpin. As an aside, other varieties of this species contain normal amounts of caulerpin and caulerpicin.

It would seem (Table 1) that *Oxynoe* contains caulerpicin and caulerpin, and in addition, like the *Caulerpa* on which it was growing, both palmitic acid and β -sitosterol. Based on proportionate dry weight of the sources, the *Oxynoe* had (Table 2) caulerpicin and caulerpin concentrations in excess of, respectively, 3.4 and 2.3 times those in *Caulerpa*. How this concentration takes place or where it occurs in the ani-

mals, we cannot decide from the present materials. Co-chromatograms of the *Oxynoe* extract with crystalline caulerpin were used to demonstrate the concentrated material to be caulerpin. Further collections should be made with gut and flesh separated, and it is hoped that when this is done the toxic exudate reported on by Lewin (1970, accompanying paper) can also be separated and examined. In the *Oxynoe* extracts, palmitic acid and β -sitosterol were proportionately in lower concentrations than in the *Caulerpa* extracts (Fig. 1). All four chemicals were

TABLE 1

RELATIVE ABUNDANCE OF THE FOUR *Caulerpa* COMPOUNDS IN *Caulerpa* SPECIES AND IN ANIMALS ASSOCIATED WITH THEM

(Each horizontally distinct data group represents a set of samples from one community, each of which was dominated by the different *Caulerpa* species named, except for the last in which there was no *Caulerpa*.)

+ = Concentrations above the proportions normal for most species of *Caulerpa*, n = the normal protection, = less than the normal, 0 = absence. The five-place numbers are those on the voucher specimens. The animal identities are discussed in the text.)

ORGANISM	COLLECTION NUMBER	CAULER- PICIN	CAULER- PIN	PALMITIC ACID	β-SITO- STEROL
<i>Caulerpa sertularioides</i>	20540	n	n	n	n
<i>Oxynoe panamensis</i>	20541	+	n	—	—
<i>Caulerpa lentillifera</i>	20450	n	n	n	n
Soft coral	20451	0	0	—	—
<i>Caulerpa lentillifera</i>	20490	n	n	n	n
Soft coral	20576	—	0	n	n
Soft coral	20577	—	0	?	?
<i>Caulerpa racemosa</i> var.	20272	—	0	n	n
Crabs	20579	0	0	n	+
Shrimp	20578	0	0	n	n
<i>Cerithium coralium</i>	20276	—	0	n	n
<i>Caulerpa lamourouxii</i>	25299	n	n	n	n
<i>Cerithium coralium</i>	20277	—	0	n	n
(No <i>Caulerpa</i>)					
<i>Cerithium coralium</i>	20278	0	0	n	n

present (Table 1) in the *Caulerpa* on which the *Oxynoe* was collected, and in their normal proportions.

Two species of soft coral bore caulerpicin (Table 1) but none contained caulerpin. The soft corals were certainly of different species, and, while palmitic acid and β-sitosterol were present in two, it was questionable whether they were present in the third species tested. The extract of the Bulusan soft coral produced one of the most dense and sharply defined TLC

spots we obtained. Having an R_f just less than caulerpin, this unidentified substance was, therefore, troublesome in the present work.

The crabs and shrimp examined seemed to show the presence of two of the substances but lacked both caulerpicin and caulerpin (Table 1). Since caulerpicin and caulerpin have deleterious and other effects on mammals (unpublished results obtained in cooperation with Dr. George W. Read and Mr. Midori Kashiwagi), including, it would seem, being allergenic to people, this lack of concentration is perhaps fortunate, for the shrimp, if not the crabs, would seem to be potentially a commercially important food organism for the Philippine people or for export.

No caulerpin was found (Table 1) in any of the three collections of *Cerithium coralium* and only in the case of those animals from the mud under *Caulerpa racemosa* var. *uvifera* and under *C. lamourouxii* were there faint spots indicating the presence of caulerpicin. In this case, special variations in thickness of the layer and ratio of the solvent mixture used in the chromatographic process were required to make sure of cauler-

TABLE 2

RELATIVE CONCENTRATION OF FOUR SUBSTANCES PRODUCED BY *Caulerpa sertularioides* AND FOUND IN THE HERBIVORE *Oxynoe panamensis*

(The values given are in terms of percent crude yield of the dry sample of *Caulerpa* or, for *Oxynoe*, as a multiple (f) of the concentration in *Caulerpa*.)

SUBSTANCE	<i>Caulerpa</i> %	<i>Oxynoe</i> f
Caulerpin	0.85	2.3
Caulerpicin	0.87	3.4
Palmitic acid	1.20	1.0
β-sitosterol	1.00	1.2

pixin's presence. Both palmitic acid and β -sitosterol were consistently present and, if anything, unusually concentrated. The lack of caulerpixin and caulerpin in *Cerithium* (20278) from the pond having no *Caulerpa* is not surprising. Though these 2-cm-long, slender-shelled gastropods might accumulate these compounds when feeding on a *Caulerpa* species containing significant concentrations of them, these animals in themselves are too small to be an attractive human food source, and so their content of these two chemicals would not be, directly at least, a human problem.

In all these cases the whole animals were ground and extracted so that the gut contents were included. Since the gut volume was small in each of the cases, the gut contents are not thought to have been responsible for the strong *Caulerpa*-chemical spots obtained. In the quantitative work with *Oxynoe* the yields in relation to the dry weight would seem to negate the gut contents as having had a significant influence on the results.

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